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Discovery of *Cyrtodactylus ziegleri* Nazarov, Orlov, Nguyen & Ho, 2008 (Reptilia, Squamata, Gekkonidae) in lava caves of Vietnam (Dak Nong UNESCO Global Geopark)

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Abstract

During explorations conducted in lava caves within Dak Nong UNESCO Global Geopark, Dak Nong Province, Vietnam, from 2020 to 2023, we collected five specimens belonging to the genus *Cyrtodactylus*. Morphological and molecular analyses identified the population as *C. ziegleri*. This finding represents an important step in documenting the herpetofauna of Vietnam's lava caves.

Keywords

COI, *C. irregularis* group, Geckos, molecular, new record

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Introduction

The *Cyrtodactylus irregularis* Smith, 1921 species group comprises 30 species distributed across Indochina (Uetz et al. 2024), inhabiting a variety of environments such as shrublands, forests, granite outcrops, and karst formations (Grismer et al. 2021). However, its presence in lava caves has not been recorded until now. Among these species, *C. ziegleri* Nazarov et al., 2008, originally described in Chu Yang Sin National Park (CYSNP), Dak Lak Province, was previously predicted to occur in Nam Nung Nature Reserve (NNNR), Dak Nong Province (Neang et al. 2020). Our study documents its first confirmed occurrence in lava caves within Dak Nong UNESCO Global Geopark (DNGP), Dak Nong Province, Vietnam.

Material and methods

Sampling

Field surveys were conducted in 13 of the 50 lava caves in Dak Nong UNESCO Global Geopark (DNGP), Dak Nong Province, Vietnam, between 2020 and 2023. The surveyed lava caves included C0, C1, C2, C3, C4, C6.1, C7, C8, P4, P6, P8, P20, and PT06 (Fig. 1). The topographical mapping of these lava caves was previously detailed by La et al. (2018) within the 15 × 15 km lava field (Laumanns 2018). We conducted surveys within lava caves during the day (8h–21h) and searched for *C. ziegleri* using the Visual Encounter Survey method (Crump and Scott 1994). When one individual was observed in lava caves, this species was considered present. Nearby the observed individuals we recorded the following environmental features: oxygen concentration (using Smart Sensor AR8100); light intensity (using GM1010 Digital Lux Meter); air temperature, relative humidity and wind speed (using Kestrel 3000). Furthermore, the noise pollution caused by human activities may have a negative effect on cave animals (e.g., bats) (Mačić et al. 2019; Piano et al. 2022). Therefore, we also measured the sound intensity in lava caves by using EXTECH 407730 Digital Sound Level Meter. Additional species co-occurring with geckos were also recorded. The complete dataset is available on *figshare* (Nguyen et al. 2024a). While the lava caves lack permanent water bodies, water dripping from the ceiling helps maintain stable humidity levels. Geckos were collected by hand, photographed, fixed in 90% ethanol for 24 hours, and stored in 70% ethanol.

Identification

Species identification was conducted based on morphological characteristics following the descriptions provided by Nazarov et al. (2008) and Ngo et al. (2024), utilizing a stereomicroscope for detailed observation. Bilateral scale counts were documented as left/right in the ventral view. Liver tissues were extracted for molecular analysis to confirm the

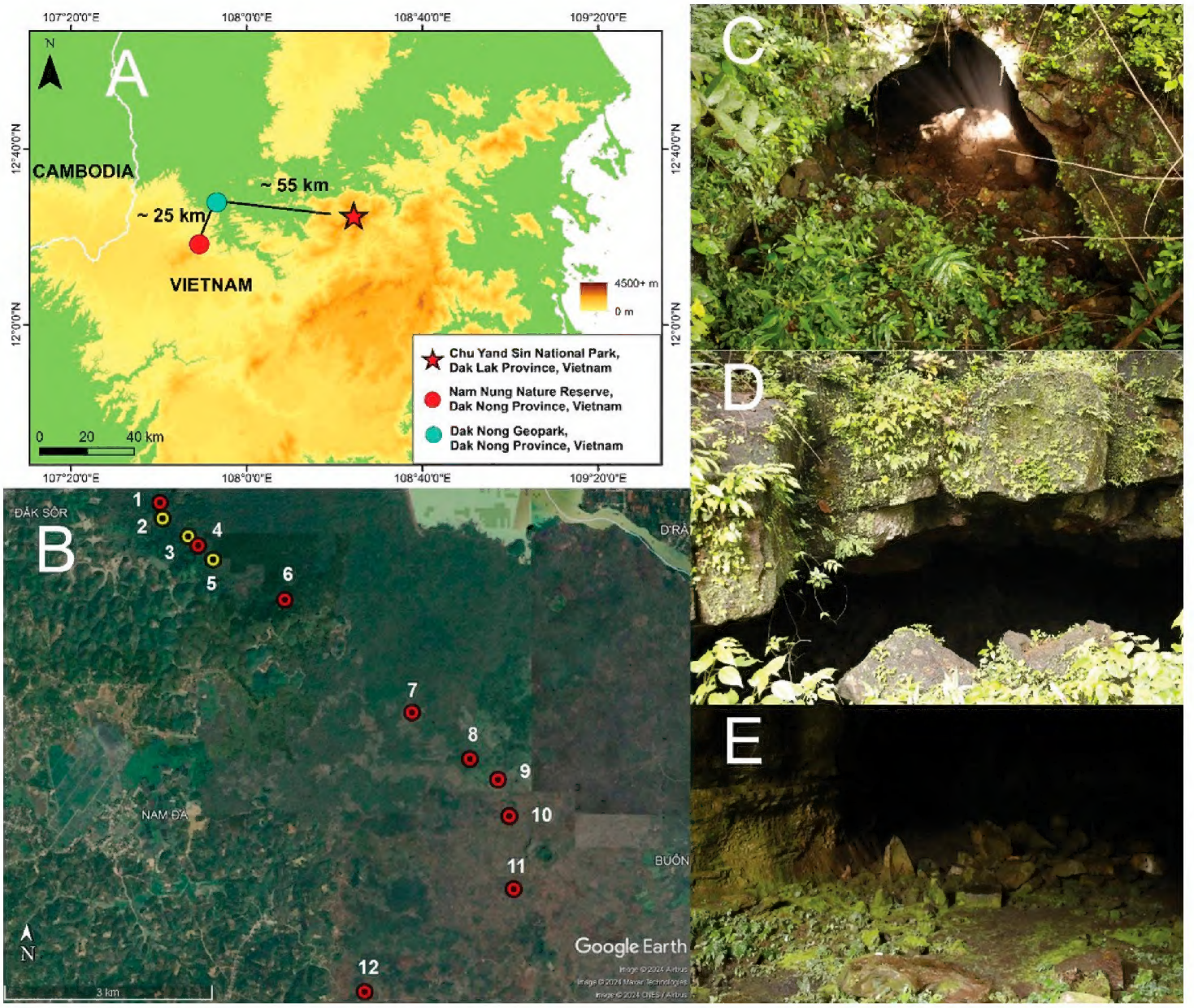

Figure 1. A known distribution of *Cyrtodactylus ziegleri* in Vietnam (star is type locality and dots are later records) **B** map of surveyed lava caves in the Dak Nong UNESCO Global Geopark (circle icon with dot), including lava caves with *C. ziegleri* records (yellow) and lava caves without records (red). Surveyed locations: 1 = cave C0; 2 = C1–C2; 3 = C3; 4 = C4; 5 = C6.1; 6 = C7; 7 = P20; 8 = P8; 9 = C8; 10 = P4; 11 = P6; 12 = PT06 **C** entrance of cave C1 **D** entrance of C3 **E** entrance of C6.1.

morphological identification and ensure accuracy. DNA extraction employed the CTAB method (Eckert et al. 2019). Fragment of the 549 base pair encoding cytochrome c oxidase subunit 1 (COI) from ITBCZ 10209 and 11035 were amplified using the primer pair VF1d - VR1d (Ivanova et al. 2007). PCR reaction followed MyFi™ DNA Polymerase kit. New sequences were inspected by CHROMATOGRAM (Chromas software version 2.6.6) then combined with all available sequences of the *Cyrtodactylus* from Neang et al. (2020) and Ngo et al. (2024) with *C. wayakonei*, and *C. spelaeus* as outgroup. The dataset was aligned using CLUSTALW (Thompson et al. 1997) then calculated p-distances in MEGA11 (Tamura et al. 2021). Phylogenetic trees were constructed using Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. The best-fit model of sequence evolution was TVMe+I+G4, TPM3+F+I and TN+F+ASC+G4 selected by MODELFINDER (Kalyaanamoorthy et al. 2017) for codon positions 1, 2 and 3, respectively. The BI analysis was implemented in MRBAYES v3.2 (Ronquist et al.

2012). Bayesian posterior probabilities (BPP) were estimated by using a Markov Chain Monte Carlo sampling method with 10,000,000 generations, saving one tree every 100 generations and were stopped when the average standard deviations reached 0.002494. The ML analysis was performed in the IQ-TREE (Trifinopoulos et al. 2016) with 1,000 bootstrap pseudoreplicates via the ultrafast bootstrap (UFB) approximation algorithm (Hoang et al. 2018). We considered nodes having BPP ≥ 0.95 and UFB ≥ 95% as being strongly supported (Hillis and Bull 1993; Minh et al. 2013).

Results and discussion

The morphology of the lava cave population largely corresponds to the type series of *C. ziegleri* described by Nazarov et al. (2008), but exhibits smaller sizes (56.0–72.8 mm vs. 84.6–93.0 mm) and slight variations in specific features, including internasal counts (1–2 vs. 1), postcloacal tubercles on each side (2–3 vs. 3), lamellae under the fourth finger (14–17 vs. 16–19), and lamellae under the fourth toe (15–19 vs. 18–21) (Nguyen et al. 2024b). These differences, however, are insufficient to classify the population as a distinct species from *C. ziegleri*.

The molecular analysis results from Bayesian Inference (BI) and Maximum Likelihood (ML) approaches confirm that the lava cave population belongs to *C. ziegleri*, with strong statistical support (BPP = 1.00; UFB = 100) (Fig. 2). The lava cave population exhibits the smallest p-distance (0.7–4.6%) from *C. ziegleri* but shows greater genetic divergence from its closest relative, *C. phnomchiensis* (6.1%). These findings suggest that the lava cave population, inhabiting 671,000-year-old lava caves (La et al. 2018), has not undergone full speciation compared to the youngest *Cyrtodactylus* lineages (~2.1 million years) (Nguyen et al. 2017).

Interestingly, the specimens from the lava caves in DNGP exhibit an average genetic divergence of 0.4%, which is higher than that observed in other *C. ziegleri* populations, such as those from CYSNP clades 1 (0.0%), clade 2 (0.2%), and the NNNR population (0.2%). This increased divergence may be attributed to limited connectivity between the cave-dwelling populations and those outside the caves.

New record

***Cyrtodactylus ziegleri* Nazarov, Orlov, Nguyen & Ho, 2008**
English and Vietnamese: Ziegler's Bent-toed Gecko and Thằn lằn ngón dít-lơ-ri

Specimens examined. Vietnam • 3 ♂♂; DNGP, Dak Nong Province, cave C1 ;12°31'11.0"N, 107°53'34.4"E; December 2020; V.H.D. Nguyen leg.; ITBCZ 10209, 10211, 11034 • 1 ♀; DNGP, Dak Nong Province, cave C3; 12°31'02.4"N, 107°53'47.3"E; October 2023; V.H.D. Nguyen, T.G. Tran leg.; ITBCZ 11035 • 1 ♀; DNGP, Dak Nong Province, cave C6.1; 12°30'51.2"N, 107°53'59.8"E; October 2023; V.H.D. Nguyen, T.G. Tran leg.; ITBCZ 11024.

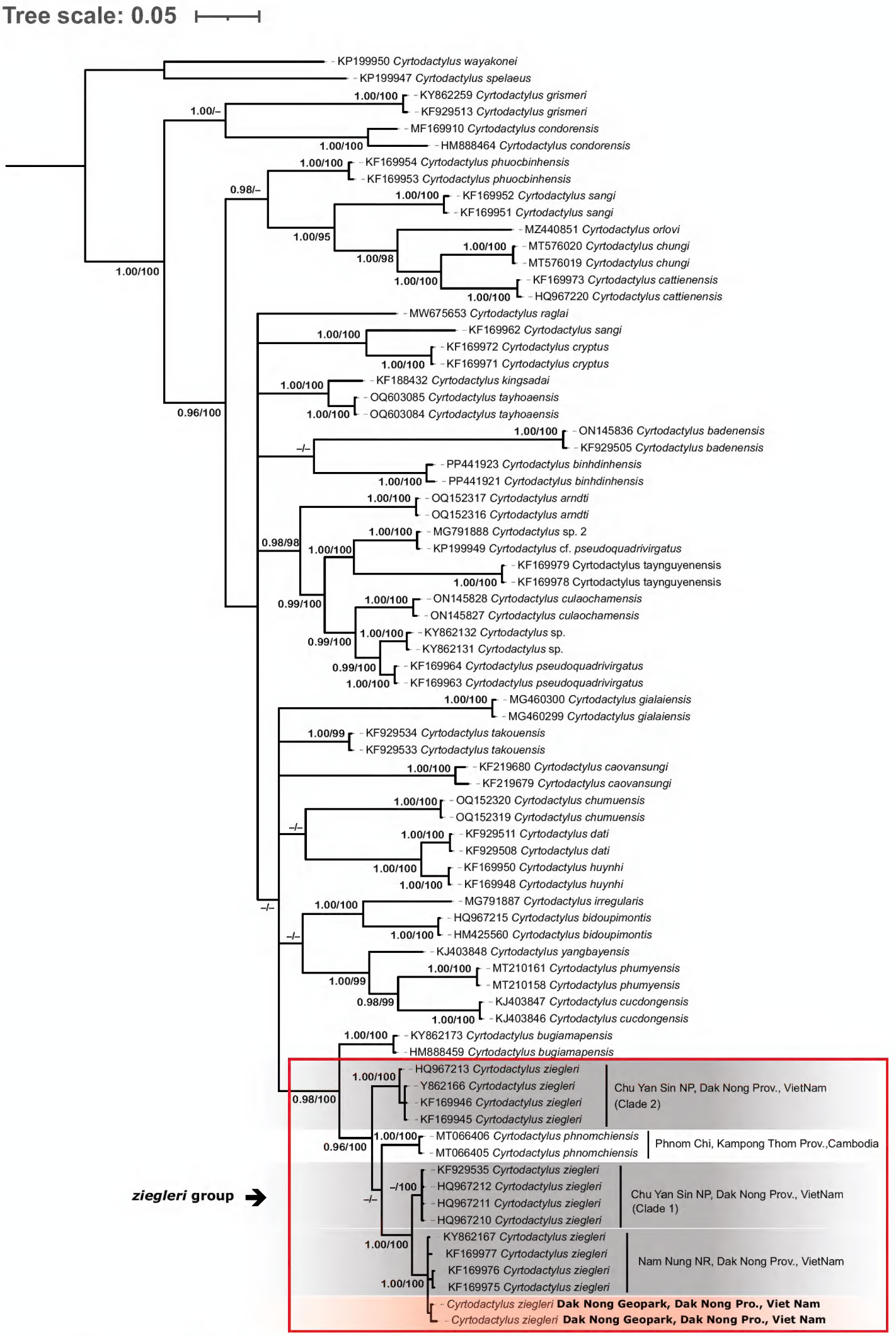

Figure 2. Bayesian inference (BI) tree for the *C. ziegleri* based on COI. Numbers left and right are ML ultrafast bootstrap (UFB)/Bayesian posterior probabilities (BPP), respectively. Dashes denote strongly supported values of BPP < 95% or UFB < 95.

2 ♂ (ITBCZ 10209, ITBCZ 10211) and 1 ♂ (ITBCZ 11034) from cave C1 (12°31'11.0"N, 107°53'34.4"E), DNGP, Dak Nong Province, Vietnam, collected December 2020 by V.H.D. Nguyen • 1 ♀ (ITBCZ 11035) from cave C3 (12°31'02.4"N, 107°53'47.3"E) and 1 ♀ (ITBCZ 11024) from cave C6.1 (12°30'51.2"N, 107°53'59.8"E), collected October 2023 by V.H.D. Nguyen, T.G. Tran.

Notes. The specimens were observed on the ceilings and within rock crevices, located 0–50 m inside the lava caves during both day and night. Cave C1 has a single entrance and extends to areas with low oxygen concentrations at its deepest survey points (17.7% at 180 m inside). The cave measures a maximum explored length of 195 m and a depth of 4.5 m. Cave C3, also with a single entrance, has a maximum explored length of 716.3 m and a depth of 7.3 m. Cave C6.1 features three entrances, with a maximum explored length of 293.7 m and a depth of 4.6 m (La et al. 2018). Microhabitat parameters recorded at the eight gecko occurrences are shown in Table 1.

Table 1. Microhabitat parameters at *Cyrtodactylus ziegleri* occurrences. / -no data.

Sample		Cave C1	Cave C2	Cave C3	Cave 6.1
		N = 3	N = 1	N = 3	N = 1
Distance To Cave Entrance (m)	Mean ± SD	23.33 ± 5.77	30	23.33 ± 25.17	15
	Min	20	/	0	/
	Max	30	/	50	/
Oxygen Concentration (%)	Mean ± SD	20.27 ± 0.21	20.2	20.63 ± 0.29	20.7
	Min	20.10	/	20.30	/
	Max	20.50	/	20.80	/
Temperature (°C)	Mean ± SD	27.20	/	26.45 ± 0.78	24.9
	Min	/	/	24.90	/
	Max	/	/	25.90	/
Humanity (%)	Mean ± SD	85.30	/	89.15 ± 10.82	100
	Min	/	/	81.50	/
	Max	/	/	96.80	/
Light Intensity Max (lux)	Mean ± SD	0.40 ± 0.69	0	25.70 ± 44.51	0
	Min	0	/	0	/
	Max	1.20	/	77.10	/
Light Intensity Min (lux)	Mean ± SD	0.37 ± 0.64	0	25.13 ± 43.53	0
	Min	0	/	0	/
	Max	1.1	/	75.4	/
Wine Speed Max (m/s)	Mean ± SD	0	0	0	0
	Min	0	/	/	/
	Max	0	/	/	/
Wine Speed Avg (m/s)	Mean ± SD	0	0	0	0
	Min	0	/	/	/
	Max	0	/	/	/
Sound Intensity Max (dB)	Mean ± SD	0	/	0	48.8
	Min	0	/	/	/
	Max	0	/	/	/
Sound Intensity Min (dB)	Mean ± SD	0	/	0	0
	Min	0	/	/	/
	Max	0	/	/	/

Figure 3. A, B *C. ziegleri* (ITBCZ 11034, 11035 respectively) in life inside lava caves **C** poaching evidence as plastic nest fragments (white arrow) inside the cave C2 **D** plastic garbage (white arrow) inside the cave C3.

Sympatric fauna recorded alongside *C. ziegleri* included bats, crickets, crabs, snails, frogs (*Fejervarya limnocharis* and *Micryletta* sp.), and other lizards (*Scincella* sp.).

During cave surveys, we identified several primary threats to the cave habitat, including garbage, fire remnants, tourism, and poaching, all of which endanger cave wildlife (Fig. 3). Fires contribute to air pollution, which negatively impacts vegetation inside the caves that serve as a food source for insects (Baker et al. 2013). This, in turn, affects insectivorous species like bats and possibly *C. ziegleri*. Additionally, the heat from fires turned on inside the cave can lower the humidity and increase temperatures of the inner environment, provoking detrimental effects on the entire ecosystem (Baker et al. 2013). Tourism has also been reported to pose multiple threats to cave ecosystems, including alterations to the microenvironment due to rising CO_2 levels, temperature shifts, noise, and the introduction of dust and microorganisms from visitors (Piano et al. 2022). Understanding these threats is crucial for the conservation of these unique ecosystems.

Conclusion

Our observation of *C. ziegleri* in lava caves within the Dak Nong UNESCO Global Geopark, located 55 km from its type locality, reveals a genetic divergence of 0.4% in cave populations. This is higher than the genetic variation observed in other popula-

tions (0.0–0.2%), suggesting limited connectivity and a gradual adaptation to the cave environment. The observed divergence indicates potential ecological isolation and ongoing adaptation to specific cave conditions. Additionally, threats such as agriculture, pollution, and tourism emphasize the urgent need for further research and conservation efforts to protect these unique habitats and their inhabitants.

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